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Comparison of G1.2/G2.2 and Sydney IVF cleavage/blastocyst sequential media for the culture of human embryos: a prospective, randomized, comparative study

[Reproductive Biology]

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Outline

Graphics

Abstract

Objective: To compare two commercially available sequential media, G1.2/G2.2 and Sydney IVF cleavage/blastocyst media, as supports for human embryo culture.

Design: Prospective randomized study.

Setting: University-based IVF clinic.

Patient(s): Two hundred forty-nine patients undergoing IVF treatment for the first or second time, randomly allocated at the time of oocyte retrieval, to either culture in G1.2/G2.2 or Sydney IVF media.

Intervention(s): Oocyte recovery, IVF or intracytoplasmic sperm injection, embryo culture, transfer on day 3 or day 5/6.

Main Outcome Measure(s): Developmental stage on day 3, blastocyst rate, pregnancy outcome as assessed by [beta]hCG positive test, implantation rates, and ongoing pregnancies.

Result(s): Embryos cultured in G1.2/G2.2 media displayed a faster kinetics of cleavage, compaction, blastulation, and hatching, but a lower day 3 embryo quality than those grown in Sydney IVF media. For patients with at least five embryos, G1.2/G2.2 media yielded higher implantation rates (26.2%) in our day 3 embryo transfer program when compared to Sydney IVF medium (15.5%), whereas similar implantation rates were obtained for day 5/6 embryo transfer for both media (43.1% and 36.1%, respectively).

Conclusion(s): In our day 3 embryo transfer program, G1.2/G2.2 media were superior to Sydney IVF media, whereas both media yielded similar outcomes in our blastocyst transfer program.

The recent development of serum-free sequential media designed for human embryo culture has led to improvements in IVF and intracytoplasmic sperm injection (ICSI) outcomes. These media were formulated according to the carbohydrate composition of oviduct and uterine fluids and take into account the changing physiology and metabolic requirements of the human embryo (1). A first glucose-poor or even glucose-free medium is designed to support the development of the zygote to the eight-cell stage, followed by a second, more complex, medium suitable for the development, after activation of the embryonic genome, up to the blastocyst stage.

An increasing number of studies have confirmed that high implantation rates can be achieved after transfer of blastocysts obtained with this new generation of media (2-9). Implantation rates of up to 50%, close to those resulting from the transfer of in vivo-derived embryos, have been reported for patients with a good prognosis (5-7). The viability of the blastocysts produced is greater than that reported for culture systems using one medium throughout the culture, such as a mixture of Earle's and Ham's F-10 (10, 11), Earle's (12), or minimal essential medium (MEM) (13). Results achieved with sequential media appear to be in the same range as those reported when embryos are cocultured with feeder cells (8, 14, 15). However, culture in sequential media offers several advantages over coculture. The risk of introducing pathogens is greatly reduced, culture handling is less time-consuming, and the composition of the culture medium is less uncertain.

Several of these sequential media are commercially available, including G1/G2 media (6, 16, 17), G1.2/G2.2 media (5, 7), S1/S2 media (3), P1/Irvine Scientific Blastocyst media (8, 18), IVF-50/G2 (2, 17), IVF 50-S2 (4, 9, 14), EllioStep2 (19), and Sydney IVF cleavage/blastocyst media or M 91 (20). However, few studies have compared the proficiency of these media and assessed the viability of day 3 embryos obtained in sequential media.

The aim of the present study was to assess pregnancy outcomes after transfer of day 3 embryos and day 5 or 6 blastocysts obtained in G1.2/G2.2 media and Sydney IVF media and to compare the in vitro development of embryos in these sequential media using developmental speed and embryo morphology as end points.

MATERIALS AND METHODS

Inclusion Criteria

The present prospective randomized study included all the cycles from patients at their first or second attempts. From June 1999 to February 2000, 249 IVF cycles were included in the study.

All the procedures were approved by the Institutional Board (Ethics Committee of the Catholic University of Louvain).

Design of the Study

At oocyte retrieval, patients were randomly (alternately) assigned to one of two groups. In group 1 (n = 122), zygotes were cultured in G1.2/G2.2 media (Scandinavian IVF Science, Gothenburg, Sweden) and in group 2 (n = 127) culture was performed in Sydney IVF cleavage/blastocyst media (Cook, Brisbane, Australia) (Fig. 1). On the day of fertilization check, if fewer than five zygotes were available (n = 45 in group 1 and n = 46 in group 2), embryos were allocated to our day 3 embryo transfer program. If more than five zygotes were obtained, patients were randomly divided (by drawing lots) between transfer on day 3 postinsemination (n = 40 in group 1 and n = 42 in group 2) or day 5/6 (n = 37 in group 1 and n = 39 in group 2). As shown in Tables 1, 2, and 3, the patient populations were similar in the different groups studied in the day 3 embryo transfer program as well as in the blastocyst transfer program. There was no difference in the age of the patient, number of previous attempts, stimulation protocol, number of mature oocytes, proportion of cycles with ICSI, and number of zygotes.

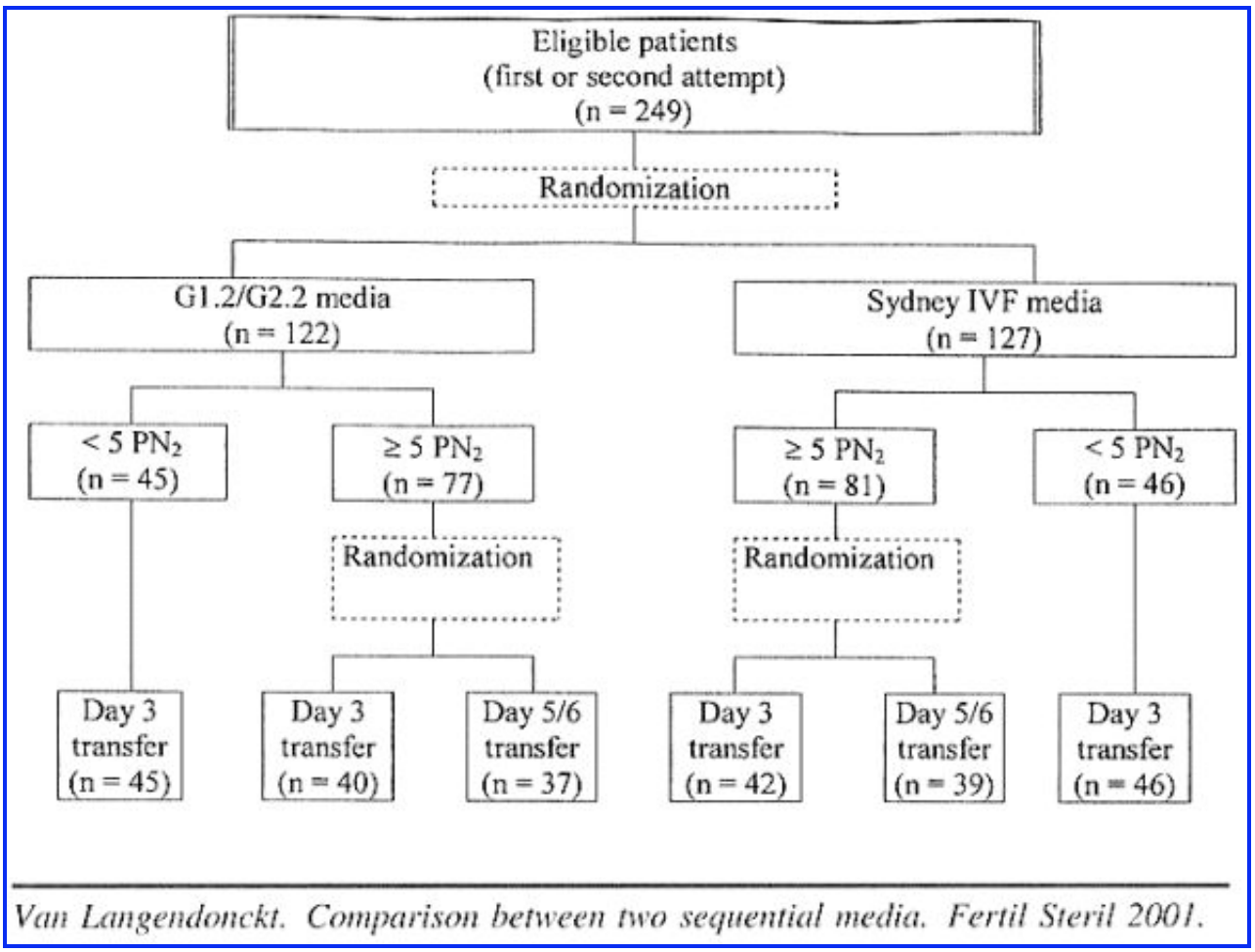

FIGURE 1 Design of the study.

Characteristics/results	G1.2	Sydney IVF	*P* value
Cycles	40	42	
Age in years (mean ± SD)	34.1 ± 5.7	33.1 ± 4.5	.1
Indications			
Male factor	10 (25.0%)	14 (33.3%)	.1
Tubal factor	8 (20.0%)	6 (14.2%)	.5
Ovarian disorders	4 (10%)	4 (9.5%)	6.0
Idiopathic	6 (15.0%)	5 (11.9%)	1.5
Endometriosis	4 (10.0%)	5 (11.9%)	2.0
Multiple factors	8 (20.0%)	8 (19.0%)	5.0
Number of previous attempts (mean ± SD)	1.6 ± 0.8	1.6 ± 1.3	
No. of patients stimulated with a long protocol (%)	32 (80%)	36 (86%)	.7
No. of mature oocytes (mean ± SD)	10.8 ± 4.8	11.7 ± 4.7	.1
No. of pronuclear embryos (mean ± SD)	8.5 ± 4.0	7.8 ± 3.7	.2
No. of cycles with ICSI (%)	26 (65%)	29 (69%)	2.0
No. of embryos transferred/cycle	2.7 ± 0.6	2.5 ± 0.8	.06
No. of cycles with embryo freezing (%)	22 (55%)	16 (38%)	<.05
Pregnancies (βhCG +)[a]	22 (55%)	17 (40.5%)	<.05
Ongoing pregnancies[a]	17 (42.5%)	13 (30.9%)	<.05
Multiple pregnancies[b]	4 (23.5%)	3 (23.1%)	5.0
Implantation rate			
No. of sacs/No. of embryos transferred	28/107 (26.2%)	21/103 (15.5%)	<.05
No. of fetal hearts/No. of embryos transferred	27/107 (25.1%)	20/103 (14.6%)	<.05

[a] Total number (% of the total number of cycles).
[b] Total number (% of the total number of ongoing pregnancies).
Van Langendonckt. Comparison between two sequential media. Fertil Steril 2001.

TABLE 1 Results of the transfer of day 3 embryos after culture in G1.2 and Sydney IVF cleavage media (for patients with at least 5 pronuclear embryos put into culture).

Characteristics/results	G1.2	Sydney IVF	*P* value
Cycles	45	46	
Age in years (mean ± SD)	34.1 ± 4.9	33.8 ± 4.0	1.0
Indications			
Male factor	18 (40.0%)	18 (39.1%)	6.6
Tubal factor	7 (15.6%)	7 (15.2%)	11
Ovarian disorders	1 (2.2%)	1 (2.2%)	11
Idiopathic	7 (15.6%)	5 (10.9%)	.7
Endometriosis	4 (8.9%)	7 (15.2%)	.06
Multiple factors	8 (17.7%)	8 (17.4%)	10.5
Number of previous attempts (mean ± SD)	1.6 ± 0.8	1.7 ± 0.8	.2
No. of patients stimulated with a long protocol (%)	27 (60%)	32 (70%)	.06
No. of mature oocytes (mean ± SD)	6.4 ± 3.8	6.0 ± 3.9	.7
No. of pronuclear embryos (mean ± SD)	2.6 ± 1.2	2.7 ± 1.1	.8
No. of cycles with ICSI (%)	36 (80%)	34 (74%)	.5
No. of embryos transferred/cycle	2.1 ± 0.7	2.2 ± 0.8	.5
No. of cycles with embryo freezing (%)	4 (8.9%)	0	
Pregnancies (βhCG +)[a]	16 (35.6%)	18 (39.1%)	2.7
Ongoing pregnancies[a]	12 (26.7%)	13 (28.3%)	5.0
Multiple pregnancies[b]	5 (18.5%)	3 (12.0%)	
Implantation rate			
No. of sacs/No. of embryos transferred	18/95 (18.9%)	17/102 (16.6%)	1.5
No. of fetal hearts/No. of embryos transferred	18/95 (18.9%)	15/102 (14.7%)	.3

[a] Total number (% of the total number of cycles).
[b] Total number (% of the total number of ongoing pregnancies).
Van Langendonckt. Comparison between two sequential media. Fertil Steril 2001.

TABLE 2 Results of the transfer or day 3 embryos after culture in G1.2 and Sydney IVF cleavage media (for patients with 4 or less embryos available for culture).

Characteristics/results	G1.2/G2.2	Sydney IVF	*P* value
Cycles	37	39	
Age in years (mean ± SD)	32.9 ± 4.5	33.5 ± 4.1	.5
Indications			
Male factor	16 (43.2%)	18 (46.2%)	3.0

Tubal factor	5 (13.5%)	4 (10.3%)	1.5
Ovarian disorders	2 (5.4%)	4 (10.3%)	.3
Idiopathic	2 (5.4%)	4 (10.3%)	.3
Endometriosis	4 (10.8%)	3 (7.5%)	1.5
Multiple factors	7 (18.9%)	6 (15.4%)	2.0
No. of previous attempts (mean ± SD)	1.3 ± 0.5	1.4 ± 0.5	.2
No. of patients stimulated with a long protocol (%)	32 (86%)	33 (85%)	2.0
No. of mature oocytes (mean ± SD)	12.9 ± 5.2	14.0 ± 4.9	.06
No. of pronuclear embryos (mean ± SD)	9.4 ± 3.1	9.9 ± 4.3	.06
No. of cycles with ICSI (%)	24 (65%)	29 (74%)	.07
Day of embryo transfer			
No. of patients with transfer at day 5	23	22	
No. of patients with transfer at day 6	14	17	
No. of embryos transferred/cycle (mean ± SD)	1.9 ± 0.4	2.1 ± 0.4	<.05
No. of cycles with embryo freezing (%)	17 (45.9%)	16 (41.0%)	1.6
Percentage of blastocysts[a]			
On day 5	38.6%	36.2%	.7
On day 6	48.2%	42.4%	<.05
Pregnancies (βhCG +)[b]	23 (62.2%)	22 (56.4%)	1.5
Day 5 transfer	14 (60.9%)	11 (50%)	.5
Day 6 transfer	9 (64.3%)	11 (64.7%)	10.0
Ongoing pregnancies[b]	18 (48.6%)	19 (48.7%)	10.0
Multiple pregnancies[c]	7 (38.9%)	10 (52.6%)	.5
Implantation rate			
No. of sacs/No. of embryos transferred	31/72 (43.1%)	30/83 (36.1%)	.07
No. of fetal hearts/No. of embryos transferred	27/72 (37.5%)	30/83 (36.1%)	5.0

[a] % of the total number of pronuclear embryos put into culture.
[b] Total number (% of the total number of cycles).
[c] Total number (% of the number of ongoing pregnancies).

Van Langendonckt. Comparison between two sequential media. Fertil Steril 2001.

TABLE 3 Results of the transfer of day 5 or day 6 embryos after culture in G1.2/G2.2 and Sydney IVF cleavage/blastocyst sequential media.

Ovulation Induction and Oocyte Recovery

Ovarian stimulation was achieved using GnRH analogues along with human menopausal gonadotropin in a long or short protocol.

For the long protocol, GnRH agonist was started between day 21 and 25 of the previous cycle. Sonography and E_2 dosage were done 3 weeks after the beginning of GnRHa administration to assess if the ovaries were at rest.

If the ovaries were at rest and the E_2 level was <30 pg/mL, hMG administration was begun 2 days later. Treatment was adapted according to the age of the patient, the number and size of follicles, and E_2 level. Human chorionic gonadotropin (10,000 IU) was administered when the E_2 level was ±200 pg/mL per follicle >=16 mm in diameter and in the presence of more than three follicles >20 mm in diameter.

After randomization, patients showing a low response after stimulation according to a long protocol as well as those older than 37 years and those having an FSH level higher than 10 mUI/mL at the third day of the cycle were included in a short stimulation protocol.

For the short protocol, the nasal spray form of Buserelin (Suprefact, Hoechst, Brussels, Belgium) was used. The patients began therapy on the first day of the cycle (900 μg daily) and continued until hCG administration. On the third day of the cycle, hMG was administered at variable doses according to each case. On the sixth day, hormonal and sonographic monitoring began, and the doses of hMG were adjusted accordingly. When the follicles reached 20 mm in diameter with an E_2 level of 150 pg/mL per follicle >15 mm, 10.000 IU of hCG was administered.

A similar number of patients were included in the long and short protocols in each subgroup as shown in Tables 1, 2, and 3.

Oocytes were collected 36 hours after hCG injection under ultrasound guidance.

IVF and ICSI

After recovery, the cumulus-oocyte complexes were placed in 20 μL droplets of fertilization medium (KSIFM, Cook) covered with mineral oil (M-8410, Sigma, St. Louis, MO). The same fertilization medium was used for both groups of patients. Sperm samples were prepared by the swim-up method or by the density gradient procedure using a 40:70:90 discontinuous silica gel gradient (Puresperm, Nidacon International AB, Gothenburg, Sweden) as described by Ng et al. (21). In case of severe oligospermia, sperm samples were diluted in fertilization medium and centrifuged at 300 × g for 15 minutes. The ICSI treatment was performed in cases of male factor indications and when poor fertilization had occurred in the previous IVF cycle. Conventional insemination was applied for all other indications.

For conventional IVF, 2-4 hours after oocyte retrieval, progressive motile spermatozoa were added to the fertilization drop at a final concentration of 100,000 spermatozoa/milliliter. In case of teratozoospermia (between 5% and 14% normal spermatozoa according to the strict criteria of Kruger and colleagues [(22)]), spermatozoa concentration was increased to 500,000 spermatozoa/milliliter.

For ICSI, 2 hours after oocyte collection, oocytes were stripped of the cumulus cells after treatment with hyaluronidase by aspirating several times through a 130-μm pipette (Flexipet, Cook). Oocytes showing first polar body extrusion were injected with a single spermatozoon as described by Van Steirteghem et al. (23). Injected oocytes were washed and transferred to 10 μL droplets of fertilization medium.

All incubations were performed at 37°C in a humidified atmosphere of 5% O_2, 6% CO_2, and 89% N_2. Culture was performed under 5% O_2 instead of atmospheric O_2, as this concentration has been shown to promote blastocyst development in humans as well as in many other species (24).

Embryo Culture

After an incubation of 18-20 hours, oocytes derived from IVF were denuded by aspiration through a 130-μm pipette. The oocytes were checked for fertilization 18-20 hours after insemination or after ICSI, and pronuclear zygotes showing two clearly distinct pronuclei were put into culture.

Culture was performed in 20 μL droplets covered with mineral oil (Sigma). Zygotes were cultured per groups of two in G1.2 (Scandinavian IVF Science) or Sydney IVF cleavage medium (K-SICM, Cook) up to day 3 (68-72 hours after insemination). Both culture media were devoid of phenol red so that the embryologists were not able to distinguish between the two media when examining culture dishes. Embryos were then regrouped and up to five embryos at a similar developmental stage were further cultured together in G2.2 (Scandinavian IVF Science) or Sydney IVF blastocyst medium (K-SIBM, Cook) as described by Jones et al. (2, 17). Seven different batches of media were used within the study period.

Embryo Quality

Day 3 embryos were graded according to the criteria described by Bassil et al. (25), but slightly modified. In this classification, grade 1 embryos are considered as top-quality embryos, with evenly shaped blastomeres, uniform cytoplasm, and no cytoplasmic fragments. Grade 2 embryos display at least one of the following abnormalities: dark cytoplasm, unequal blastomeres, or cytoplasmic

fragments making up less than 20% of the embryo perivitelline space. Grade 3 embryos may show between 20% and 50% of cytoplasmic fragments, and grade 4 embryos more than 50% of fragments. Day 3 embryos, which looked like a compact cellular mass with blastomeres in close contact that could no longer be counted and with blurred cellular outline, were classified as compacting (14, 26), as illustrated in Figure 2. Blastocysts stage was assessed according to the classification of Gardner and Schoolcraft (27); score 1: early blastocyst showing a nascent blastocoel cavity that is less than half of the volume the embryo; score 2: blastocyst showing a blastocoel greater than half the volume of the embryo; score 3: full blastocyst, the blastocoel completely fills the embryo; score 4: expanded blastocyst, the blastocoel volume is larger than that of the early embryo and the zona is thinning; score 5: blastocyst hatching out of its zona pellucida; score 6: fully hatched blastocyst, the blastocyst has completely escaped from its zona.

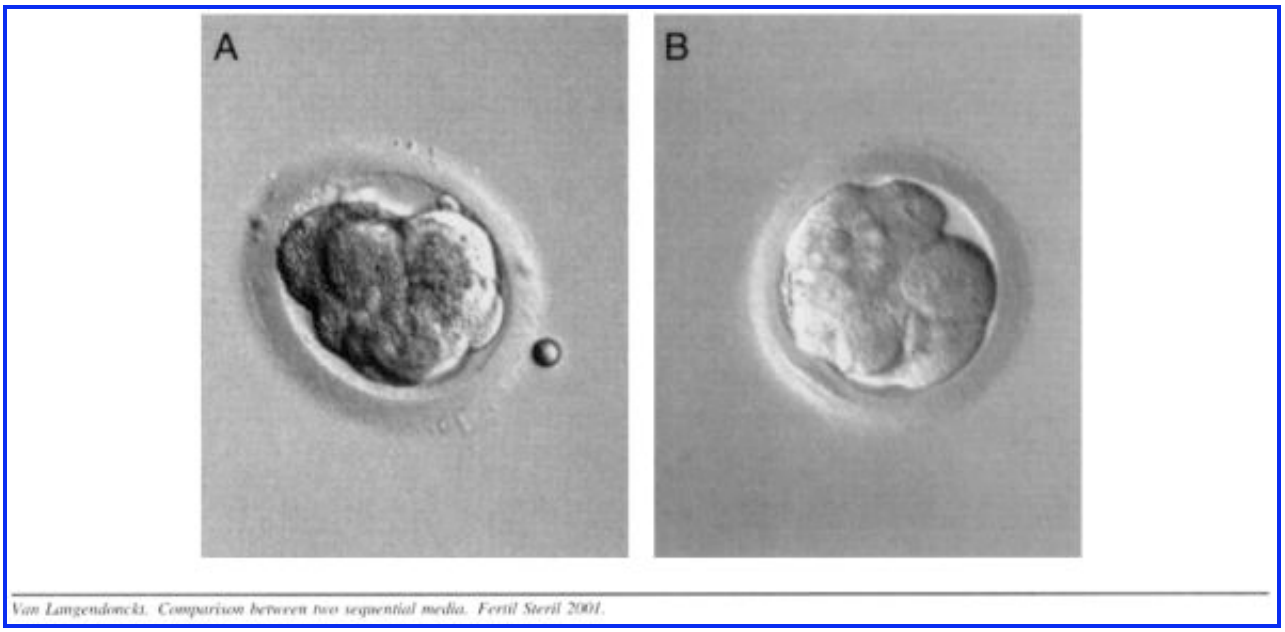

FIGURE 2 Photomicrograph of day 3 compacting embryos obtained (**A**) in G1.2 medium and (**B**) in Sydney IVF cleavage medium.

Embryo Transfer and Pregnancy Assessment

For day 3 transfers, two or three embryos (average 2.6) were selected for transfer depending on the morphological score and stage of the embryos, as well as the age of the patient. For day 5 and day 6 transfer, 1 to 3 blastocysts (average 2.2) were transferred according to their stage of development, their quality and the age of the patient. The most expanded blastocysts, clearly showing an inner cell mass (ICM), tightly packed with many cells, were preferentially selected for transfer (27). When no expanded blastocysts were available for transfer on day 5, transfer was postponed until day 6. If no blastocysts had developed by day 6, noncavitating embryos were transferred. Transfer was performed in 10 μL droplets of culture medium loaded into a Trans Soft catheter (Cook). Cryopreservation of supernumerary embryos was performed on the day of transfer if at least two embryos with good morphological scores (grade 1 or 2) were available.

A first serum hCG assay (>20 mIU/mL considered positive) was performed at least 15 days after oocyte puncture. Clinical pregnancy was determined by a weekly increase in serum [beta]hCG concentrations and confirmed by the presence of a gestational sac, crown rump length, and fetal heart beat at ultrasound performed 6 weeks after embryo transfer.

Statistical Analysis

Results are expressed as mean ± SD. Data were analyzed using the unpaired *t*-test and percentages were compared by $[chi]^2$ analysis. $P<.05$ was defined as statistically significant.

RESULTS

Day 3 Embryo Quality

As shown in Figure 3, cleavage occurred faster in G1.2 medium than in Sydney IVF cleavage medium. A significantly ($P<.05$) higher proportion of embryos had reached the eight-cell stage or were beyond this stage by day 3 (68-72 hours after insemination) in G1.2 medium (45.5%) as compared to Sydney IVF medium (36.3%), whereas a higher proportion of embryos were still at the five- to seven-cell stage in Sydney IVF (30.9%) compared to G1.2 medium (43%). Compaction also occurred earlier in G1.2 medium as indicated by a significantly higher proportion of compacting embryos on day 3 in G1.2 medium (9.5%) than in Sydney IVF medium (4.8%). A similar proportion of embryos showed delayed development (<=4 cells) in both media.

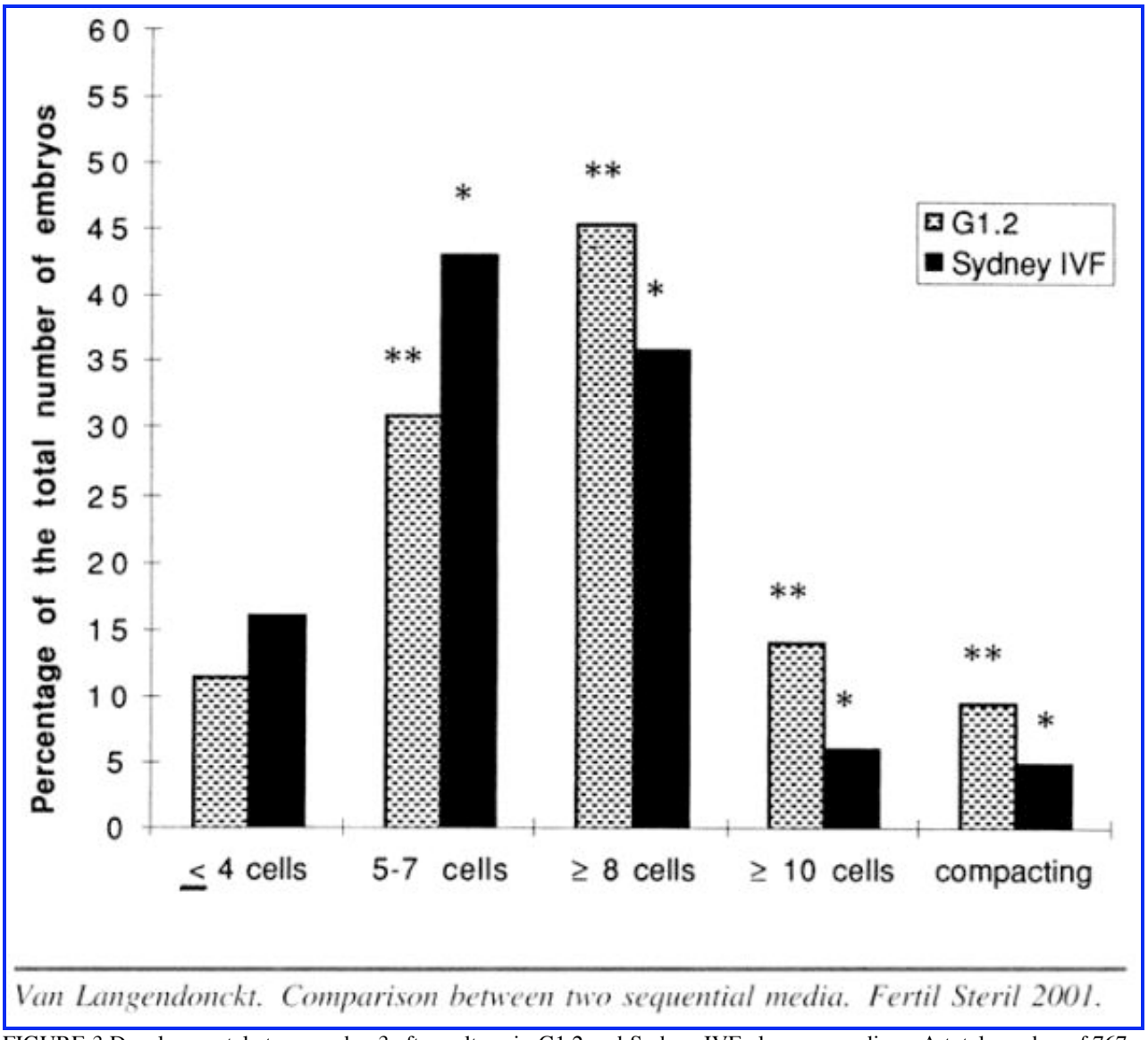

FIGURE 3 Developmental stage on day 3 after culture in G1.2 and Sydney IVF cleavage medium. A total number of 767 embryos were examined in G1.2 medium and 756 embryos in Sydney IVF medium. *Significantly different ($P<.05$) between G1.2 and **Sydney IVF medium.

(Fig. 4). The quality of day 3 embryos as assessed by their morphological appearance was superior in Sydney IVF medium than in G1.2 medium (Fig. 4). A similar proportion of grade 1 embryos (16.1% in G1.2 and 12.9% in Sydney IVF medium, respectively, P=.1) showing no abnormalities was obtained with both media but higher proportions (P<.05) of fragmented embryos with more than 20% fragmentation (score 3) or even more than 50% fragmentation (score 4) (13.0% score 3 and 6.5% score 4) were found after culture in G1.2 compared to Sydney IVF medium (8.8% score 3 and 3.1% score 4).

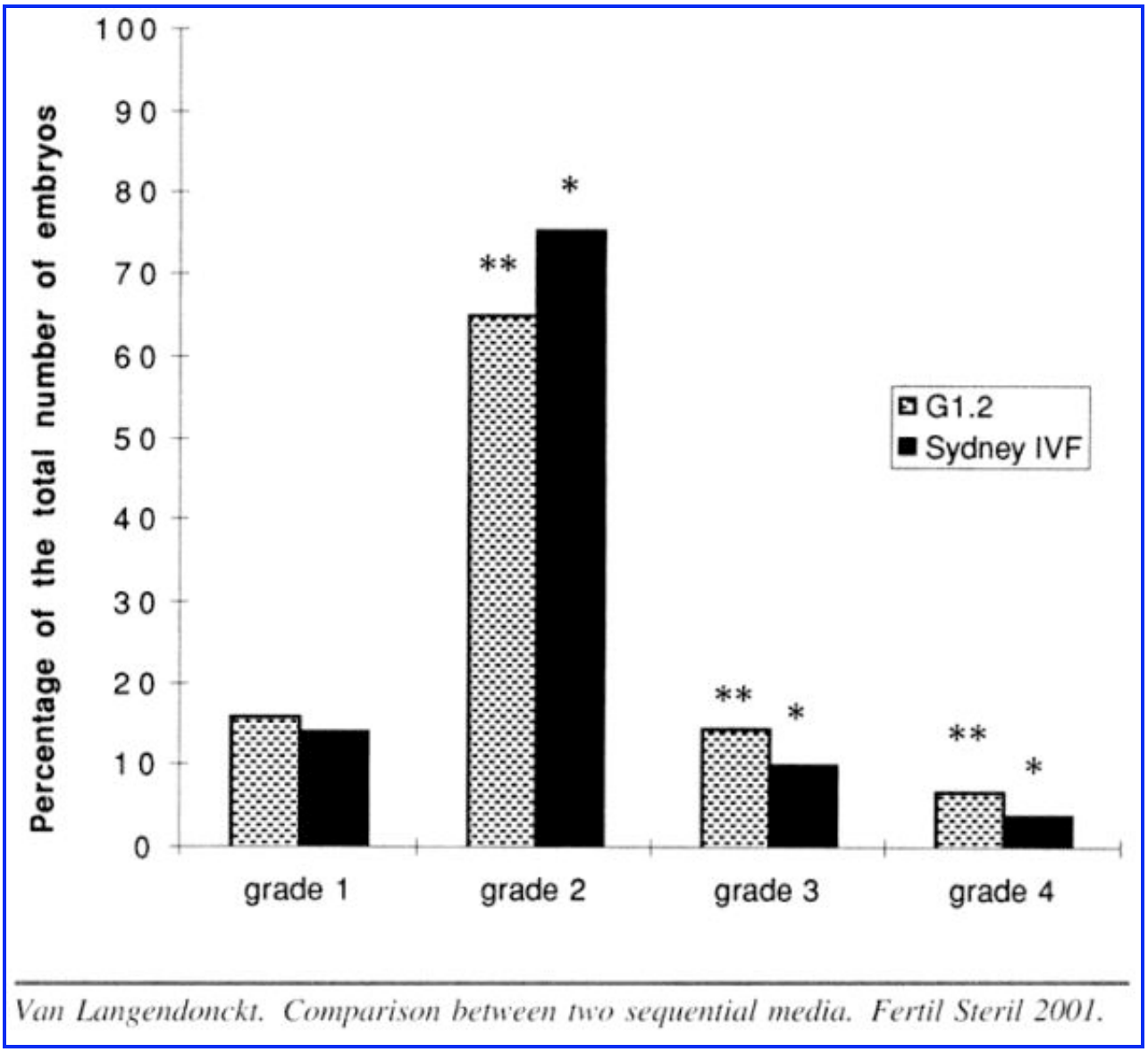

FIGURE 4 Embryo quality on day 3 after culture in G1.2 and Sydney IVF cleavage media. A total number of 767 embryos were examined in G1.2 medium and 756 embryos in Sydney IVF medium. *Significantly different (P<.05) between G1.2 and **Sydney IVF.

Pregnancy Outcomes After Day 3 Embryo Transfer

In the group of patients with at least five zygotes available for culture, transfer of day 3 embryos cultured in G1.2 medium (n = 40) resulted in higher ongoing pregnancy rates (42.5% vs. 30.9%) and implantation rates (25.1% vs. 14.6% of fetal heart beats per embryo transferred) than those obtained in Sydney IVF cleavage medium (n = 42) as shown in Table 1. The incidence of multiple pregnancies was similar with both media (23.5% in G1.2 medium and 23.1% in Sydney IVF medium, P=.6). In 55% of cycles with G1.2 medium and 38% of cycles with Sydney IVF medium, at least two high-quality

embryos were frozen (*P*<.05).

Our population of patients included a high proportion (n = 91, 37%) of women with four or less embryos (average 2.6) available for culture (37% in the G1.2 group and 36% in the Sydney IVF group) who were necessarily included in our day 3 embryo transfer program. In this group of women, pregnancy outcomes were similar with both media (Table 2). The ongoing pregnancy rate dropped to 26.7% in G1.2 medium and 28.3% in Sydney IVF medium and the implantation rate to 18.9% in G1.2 medium and 14.7% in Sydney IVF medium (*P*=.3).

When compacting embryos were available on day 3, they were given priority for transfer. Similar pregnancy rates were achieved with transfer of both compacting embryos and early cleavage stage embryos and there was no relationship between the number of compacting embryos at day 3 and the pregnancy ([beta]hCG positive) rate (data not shown).

Blastocyst Formation, Expansion, and Hatching

As summarized in Table 3, a significantly (*P*<.05) higher proportion of pronuclear embryos reached the blastocyst stage by day 6 in G1.2/G2.2 media (48.2%) as compared to Sydney IVF media (42.4%). Blastocysts were obtained in 97.2% of cycles in G1.2/G2.2 media and 91.1% of the cycles in Sydney IVF media (*P*=.06). Most blastocysts appeared on day 5 or earlier: 38.6% (80% of all blastocysts) in G1.2/G2.2 media and 36.2% (85% of all blastocysts) in Sydney IVF cleavage/blastocyst media.

Blastocyst morphological scores on the day of transfer were more advanced in G1.2/G2.2 medium than in Sydney IVF medium. When transfer was performed on day 5, a higher proportion (*P*<.05) of embryos were expanded or fully expanded (15.9%) in G1.2/G2.2 media and had started hatching (3%) compared to Sydney IVF media (12.3% and 0.8%, respectively), whereas a higher rate of embryos were at the early blastocyst or blastocyst stage in Sydney IVF media (23.1%) compared to G1.2/G2.2 media (19.7%). When transfer was postponed until day 6, 13.9% of the embryos were at the early blastocyst or blastocyst stages, 19.7% were expanded or fully expanded blastocysts, and 14.6% were hatching or had hatched in G1.2/G2.2 media vs. rates of 15.7%, 21.4%, and 5.2%, respectively, in Sydney IVF media (*P*=1.5, 2.5, and <.05, respectively).

Pregnancy Outcomes After Blastocyst Transfer

When transfer was performed at the blastocyst stage, similar implantation rates were achieved with G1.2/G2.2 media (37.5%) compared to Sydney IVF cleavage/blastocyst media (36.1%). Similar ongoing pregnancy rates was obtained with both media (48.6% in G1.2/G2.2 media and 48.7% in Sydney IVF media) despite of the fact that fewer blastocysts were transferred after culture in G1.2/G2.2 media (1.9) than in Sydney IVF media (2.1). This difference in the number of blastocyts transferred probably reflects the difference in blastocyst quality. Results were similar whether transfer was performed on day 5 or day 6. The incidence of multiple pregnancies was also similar with both media : 4 twin and 3 triplet pregnancies resulted from the transfer of blastocysts obtained with G1.2/G2.2 media (38.9% of ongoing pregnancies) and 9 twin and 1 triplet pregnancy from blastocysts obtained with Sydney IVF media (52.6% of ongoing pregnancies).

DISCUSSION

The present randomized prospective study shows that in our day 3 embryo transfer program, culture in G1.2 medium yielded faster developing embryos with a higher implantation potential than Sydney IVF cleavage medium. When culture was prolonged to the blastocyst stage, similar pregnancy outcomes were achieved with both G1.2/G2.2 and Sydney IVF cleavage/blastocyst sequential media. All the

variables that might have had an impact on our IVF results, such as the age of the patient, the number of previous attempts, the ovulatory response, the proportion of cycles with ICSI, and the number of mature oocytes and zygotes available for culture were similar with the two media in both our day 3 embryo as well as our blastocyst transfer programs.

The most striking difference between the two culture systems was a discrepancy in developmental kinetics and embryo quality on day 3. Embryos cultured in G1.2 medium appeared to cleave slightly faster than those grown in Sydney IVF cleavage medium, as indicated by a higher proportion of embryos with >=8 cells, >=10 cells, and compacting embryos on day 3. The proportion of embryos (46% in G1.2 medium) that developed to the eight-cell stage or beyond by day 3 was in the same range as the value reported for IVF-50 medium (2). Up to 9.5% of the embryos had already started compacting by day 3 in G1.2 medium, which is in agreement with the findings of Fong and Bongso (14) who observed 10% of day 3 embryos compacting in IVF-50 medium. Intriguingly, in their report, after inclusion of Vero cells in IVF-50 as coculture support, 19% of embryos were observed compacting on day 3 as compared to 10% in medium alone. On the contrary, compaction seems to occur later in Earle's medium supplemented with serum where there was no evidence of compaction in human embryos up to day 4 (26). However, further studies are required to evaluate whether this earlier compaction in G1.2 medium has an impact on further embryo development, as compaction can occur with a limited number of cells and it has been suggested in the bovine that alteration in compaction may lead to disturbed ratio between ICM and trophectoderm (28). In the present study, we found no relationship between the proportion of compacting embryos and pregnancy outcomes.

This difference in early developmental kinetics is difficult to interpret as the exact composition of the media is not disclosed. One main difference between G1.2 and Sydney IVF cleavage medium seems to be the complete absence of glucose from the latter. During the early cleavage stages, embryos can be grown in simple media devoid of glucose (8, 18, 19, 29). However, the complete removal of glucose from culture media may be regarded as nonphysiological as glucose has been found in the oviduct and glucose transporters are expressed in early cleavage stage embryos as pointed out by Gardner and Lane (1) and by Ménézo et al. (15). Barak et al. (29) observed a faster rate of development on day 3 in media containing glucose compared to glucose-free medium, which is consistent with the results obtained in the present study. Differences in amino acid composition may also influence the timing of first cleavage division as shown in the mouse (1). However, further studies are needed to better assess the impact of culture components on the rate of first cleavages.

Developmental kinetics can be considered as a marker of embryo quality as discussed in Gardner and Lane (1) and Bavister (30). Unfortunately, few studies have examined this parameter during human embryo development. Studies performed on the mouse and pig have shown that the fastest cleaving embryos have the highest developmental potential. This also seems to hold true in humans, as recent studies have shown that the number of eight-cell embryos observed on day 3 is a good indicator of the pregnancy outcome on day 5 (2, 4). However, selection of fast cleaving human embryos may also have an impact on the sex ratio in favor of males, as discussed by Veiga et al. (31), and should be carefully followed up.

Interestingly, day 3 embryos cultured in Sydney IVF cleavage medium had a better morphological aspect and showed a lower fragmentation rate than those cultured in G1.2 medium. Despite their superior embryo quality, in the group of patients having at least five zygotes, transfer of early cleavage stage embryos produced in Sydney IVF medium resulted in lower implantation rates than those obtained in G1.2 medium. Consequently, embryo morphology does not seem to be a reliable marker to compare the efficiency of two culture systems. Several studies have demonstrated that day 3 embryo morphology has limited predictive value for subsequent development (11).

However, in the group of women with less than five pronuclear embryos, pregnancy and implantation rates were similar with both media. This may reflect the fact that most transfers were nonselective and that embryo quality might have a higher impact on transfer results in this group.

Few studies have examined the viability after transfer of day 3 embryos obtained with medium designed for sequential culture. Clinical pregnancy rates of around 50% and implantation rates of about 20% are reported in the literature for day 3 transfers using these types of media (4, 19). The use of these media may also be beneficial for patients showing a poor response to stimulation treatment. In the present study, a pregnancy rate ([beta]hCG positive) of 35.6% in G1.2 medium and 39.1% in Sydney IVF cleavage medium was achieved for patients having four or less zygotes available for culture. Results achieved in day 3 embryo transfer programs may well be boosted by the use of sequential media as culture support, together with the recent finding of more reliable markers of day 3 embryo quality (32).

The difference in developmental kinetics was also visible on day 5, because a higher proportion of the embryos were at the expanding or expanded blastocyst stage and had started hatching in G1.2/G2.2 media on day 5. Most embryos started blastulation by day 5 in both media, as previously observed in G1/G2 media (5, 6). The viability of the blastocysts obtained in G1.2/G2.2 and Sydney IVF cleavage/blastocyst media was similar, as indicated by a similar rate of implantation. Results achieved in the present study are in the same range as those reported for the transfer of blastocysts obtained in sequential media, (with a 42%-55% pregnancy rate and a 19%-33% implantation rate) (3, 4, 8, 9, 17).

Although the present study was not designed to compare the transfer outcome of early cleavage stage embryos and blastocysts, higher implantation rates were achieved after blastocyst transfer compared to day 3 embryo transfer, as generally reported for sequential media (3, 5, 6). Although, some studies suggest that blastocyst transfer should be restricted to a selected population of patients (4, 33) and the advantage of transferring blastocyst instead of day 3 embryos is still a matter of debate. The incidence of multiple pregnancies remained high (27%) despite the fact that only 1.9 blastocysts were transferred on average after culture in G1.2/G2.2 media, suggesting that the number of blastocysts transferred can still be decreased. A recent study by Gardner et al. (7) showed that single blastocyst transfer can yield high pregnancy rates for patients with at least one top-scoring blastocyst.

In conclusion, this is one of the first large-scale studies comparing two sequential media, differing in carbohydrate and amino acid composition, for the culture of human embryos. It emphasizes the advantage of analyzing the kinetics of early cleavages and blastocyst expansion. The data presented here appear to show that, in our culture conditions, first cleavages occurred faster and the implantation potential of day 3 embryos was higher in G1.2/G2.2 media than in Sydney IVF media. When culture was prolonged to the blastocyst stage, higher implantation rates were achieved and both media yielded similar pregnancy outcomes.

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